PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 08 November 2000 (08.11.00)	
International application No. PCT/IL00/00196	**Applicant's or agent's file reference** 124100.9 SZ
International filing date (day/month/year) 29 March 2000 (29.03.00)	**Priority date** (day/month/year) 30 March 1999 (30.03.99)
Applicant PALTIELI, Yoav et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

19 October 2000 (19.10.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer R. E. Stoffel
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

COURTESY COPY OF THE

INTERNATIONAL

PRELIMINARY

EXAMINATION REPORT

ANNEXES ARE ATTACHED

BUT ARE NOT TO BE USED

FOR INITIAL EXAMINATION

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
124100.9 SZ		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IL00/00196	29/03/2000	30/03/1999
International Patent Classification (IPC) or national classification and IPC C07K16/18		
Applicant DIAGNOSTIC TECHNOLOGIES LTD. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
19/10/2000	26.06.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Herrero, M Telephone No. +49 89 2399 8542

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/IL00/00196

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-15	as originally filed				

Claims, No.:

14,15	as originally filed				
1-13	as received on	05/06/2001	with letter of	31/05/2001	

Drawings, sheets:

1/18-18/18	as originally filed

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   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
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4. The amendments have resulted in the cancellation of:

☐ the description, pages:
☒ the claims, Nos.: 6 and 12 as originally filed
☐ the drawings, sheets:

5. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)
see separate sheet

6. Additional observations, if necessary:
see separate sheet

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-13
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-13
Industrial applicability (IA)	Yes:	Claims	1-13
	No:	Claims	

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

SECTION I

5. The amendments filed with the letter dated 31.05.01 introduce subject-matter which extends beyond the content of the application as filed, contrary to Article 34(2)(b) PCT. The amendment concerned is the following (underlined):

In present Claim 1, the intended characterization of the monoclonal antibody of interest as "A monoclonal antibody (Mab) isolated using a two-Mab sandwich enzyme-linked immunosorbant assay (ELISA) and capable of binding Placental Protein 13 (PP-13)".

According to the supporting description, the hereby characterized anti-PP-13 Mabs capable of binding with high affinity/specificity PP13 (i.e. the Mabs obtainable from one of the following hybridoma clones: # 26-2 having the Accession No. I-2134, # 27-2-3 having the Accession No I-2135, # 215-28-3 having the Accession No. I-2136, # 534-16 having the Accession No. I-2137 or # 606-8-11-67 having the Accession No. I-2138) were identified/selected by means of sequential screening assays involving (i) an antibody capture ELISA (cf page 8, lines 27-29 bridging over page 9, lines 1-8 and page 11, lines 8-16) followed by (ii) a two antibody sandwich ELISA employing a rabbit polyclonal anti-PP-13 IgG as a primary Ab and antisera of test bleeds, hybridoma culture supernatants or ascitic fluids as the secondary Abs (cf page 9, lines 12-19 and page 11, lines 17-24).

The selected Mabs thus identified (i.e. the intended Mabs according to present Claim 2) were therefore not "isolated using a two-Mab sandwich enzyme-linked immunosorbant assay (ELISA)". Accordingly, the amendment introduced in the newly filed Claim 1 contravenes Article 34(2)(b) PCT.

In connection to the foregoing it is noted that, subsequently to their identification/isolation, the potential applicability of several Mabs of interest (i.e. of the intended Mabs according to present Claim 2) as components of a two-Mab sandwich ELISA for the PP13 was tested. As stated in the supporting description on page 12, lines 13-16 "*Two-antibody sandwich assays with different combinations of primary and secondary Ab were carried out. The most effective*

variant was found to use IgG # 27-2-3 for coating and Biotin-IgG # 215-28-3 as a secondary Ab (Fig. 11)" (see also page 9, lines 21-29 bridging over page 10, lines 1-22).

6. Additional observations

This preliminary examination report takes into consideration the content of the Applicants' letter of 31.05.01 in reply to the written opinion dated 04.01.01, as well as the additional technical information enclosed therein.

SECTION V

2. CITATIONS AND EXPLANATIONS

2.1 In view of the priority documents pertaining to the present application, the international patent application WO 99/38970 (publication date 05.08.99) and the scientific publication by Than, N.G. *et al* in Placenta **20**:703-710 (November 99), cited in the International Search Report under the "P" category, are not to be regarded as state of the art according to Rule 64 (1) PCT as the date of priority of 30.03.99 is hereby validly claimed.

Nevertheless, the aforementioned document WO 99/38970 (filed on 21.01.99), which describes monoclonal antibodies falling under the scope of present Claim 1 (cf page 4, lines 1-3), is brought to the Applicant's attention in view of the provisions of Article 54(3)(4) EPC.

2.2 The following documents have been considered for the purposes of this report:

D1: US-A-5198366 (also cited in the application)
D2: US-A-4500451 (also cited in the application)

D1 discloses a radioimmunoassay (RIA) and an enzyme-linked immunosorbent assay (ELISA) based on the use of labelled human placental protein 13 (PP13) and anti-PP13 polyclonal antiserum, respectively. Both assays were developed

with the purpose of enabling the detection of three specific pregnancy-related disorders: intrauterine growth retardation, preeclampsia and preterm delivery (cf column 2, lines 57-63; column 3, lines 45-68-column 4-column 5, line 1; Claims 2 and 7).

The diagnostic significance of PP13 had been previously established, e.g. in D2 (cf column 4, lines 36-47). In addition to the data related to the isolation and characterization of human PP13, D2 also teaches the immunological determination of PP13 based on the use of monospecific antisera prepared by immunizing animals (e.g. rabbits) by known methods using the purified PP13 obtained by the procedure therein disclosed (cf column 4, lines 2-22).

2.3 The newly filed Claims 1 to 13 appear to relate to subject-matter which is novel over the available prior art, as required by Art. 33(2) PCT.

The arguments defending the non-obviousness of Claims 1 to 13 put forward by the Applicants in the aforementioned letter of 31.05.01 have been taken into account. However it is still considered that the application does not satisfy the criterion set forth in Art. 33(3) PCT because the subject-matter presently claimed does not involve an inventive step (Rule 65(1)(2) PCT).

In view of the related prior art (e.g. D1 and D2 above), the objective problem solved by the claimed invention according to Claim 1 (Mabs), Claims 4-8 (immunoassays) and Claims 9-13 (kits) merely relates to the provision of Mabs raised against PP13 and kits containing the same, and their use in an immunoassay for measuring the level of PP13 in biological samples (see also page 3, lines 2-5 of the application).

To serve the above purpose the application characterizes (and seemingly renders available by means of deposit) five different Mabs. These Mabs are identified in the description (and in Claims 2 and 3) by the arbitrary numerical denominations assigned to the specific producing clones which are listed in the Table shown on page 3 (accession Nos. corresponding to deposited hybridomas producing each one of the five Mabs are additionally shown in said Table).

Taking into account the technical advances in the fields of immunology and/or biotechnology achieved at the priority date of the present application, it is apparent that the preparation of Mabs directed against the well-known protein antigen PP13, which had been already purified and used for the generation of specific polyclonal antibodies (see e.g. D1 and D2), would have not required from the person skilled in the art the practice of any inventive activity.

In the absence of evidence demonstrating the contrary it has to be assumed that, since the successful generation of monospecific antisera/polyclonal antibodies against PP13 had been previously disclosed, the preparation of corresponding Mabs (i.e. the Mabs of Claim 1) would have been obvious to the skilled person. Thus no inventive contribution is recognisable in claiming the generic Mab capable of binding PP13 according to Claim 1.

As a consequence of the above major objection Claims 4-8, encompassing conventional immunoassay procedures for measuring the level of PP-13 in a biological fluid (i.e. under standard sandwich ELISA formats employing two Mabs), all of them relying on the use of the non-inventive Mabs of Claim 1, are also objected to under Art. 33(3) PCT due to lack of inventive step.

Moreover, insofar as the packaging of the components necessary for the performance of a known or obvious assay (e.g. the ELISA of D1) into a kit is an obvious application of such an assay, present Claims 9-13, directed to kits for measuring the level of PP13 in a biological fluid, which rely of the use of the non-inventive Mabs of Claim 1 [as components (a) and (b) thereof] cannot be considered inventive, contrary to Art. 33(3) PCT.

With regard to the five specific Mabs obtainable from the deposited hybridoma cell lines with the Accession Nos. I-2134 to I-2138, it is presently not evident on which grounds they (i.e. all of them) should *per se* be acknowledged as inventive.

On the one hand, the preparation of generic Mabs directed against PP13 is considered to be straightforward for the skilled person, vis-à-vis D1 or D2 (see

above discussion). On the other hand, having regard to the supporting description on page 11, lines 7-29 bridging over page 12, lines 1-5, the selection of the five specific clones producing the aforementioned Mabs of interest seems to have been exclusively based on standard sequential screening procedures aimed at identifying several stable clones producing high affinity Abs (see the third paragraph of item 5 in the above Section I). These screening procedures, which were carried out under conventional conditions and making use of well-known components, i.e. the purified antigen PP13 and a rabbit anti-PP13 polyclonal Ab (apparently equivalent to the first Ab employed in D1), merely represent a trial-and-error approach which comes within the scope of the customary practice followed by persons skilled in the art, especially as the advantages thus achieved, i.e. the identification of particular clones producing Mabs potentially useful to carry out an improved version of the ELISA assays of D1, can readily be foreseen.

From the results presented in Figures 4, 5 and 11 it could be concluded that only the Mab produced by the selected clone denominated # 215-28-3 would be suitable to solve the underlying technical problem of efficiently differentiating in a biological fluid of interest (e.g. blood serum) those distinctive levels of PP-13 characteristic of/associated with pregnancy/pregnancy disorders (cf page 14, lines 18-24 bridging over page 15, lines 1-2). Conversely, it is not evident from the results presented in Figures 4, 5 and 11 that the rest of the specific Mabs claimed in Claim 2 could solve the problem posed.

Under these circumstances, the subject-matter of Claims 2 and 3 would not appear to satisfy the requirements of Art. 33(3) PCT as these claims respectively encompass every one of the aforementioned five specific Mabs and their corresponding producing hybridomas.

2.4 Notwithstanding the foregoing it would appear that the application contains subject-matter which is novel and inventive over the prior art and industrially applicable, as required by Art. 33(2)(3) and (4) PCT. In this regard the application seemingly enables a highly sensitive (i.e. 0.05 ng/ml of PP-13) two-Mab sandwich ELISA (cf page 12, lines 12-17) based on the use of IgG # 27-2-3 for coating (primary Mab) and Biotin-IgG # 215-28-3 as a secondary Mab (it is assumed that the advantageous results concerning sensitivity and specificity of the two-Mab

sandwich ELISA test presented in Figures 13 and 14 have been obtained employing said combination of primary and secondary Mabs, wherein the second Mab is produced by the selected clone denominated # 215-28-3).

(It is also assumed that the "Mab of the invention" employed to generate the comparative data provided by the Applicants is either the Mab produced by the selected clone denominated # 215-28-3 or a anti-PP-13 Mab with equivalent behaviour).

SECTION VII

1. The terms " Tween " (page 6, line 11 and page 10, lines 4 and 5), and "Superdex" and "Hiload" (page 7, line 14) appear to be registered trade marks, but have not been acknowledged as such.

2. The expression "hereby incorporated by reference" in respect of prior art documents on page 1, lines 14-15 and page 2, lines 3-4, leads to a doubt as to whether the requirements of the description being self-contained are satisfied (see PCT Guidelines C-II, 4-17).

SECTION VIII

The arbitrary numerical denominations given in Claims 2 and 3 to the intended hybridoma cell clones therein referred to are *per se* devoid of technical significance and leave the reader in doubt as to the actual meaning of the essential characterizing feature to which it refers, thereby rendering the definition of the subject-matter of said claims unclear (Article 6 PCT).

In order to overcome this deficiency (each one of) the clones mentioned in Claims 2 and 3 should have been clearly identified reciting, for instance, "... hybridoma ... // ... selected from the group of clones # 26-2 having the Accession No. I-2134, 27-2-3 having the Accession No I-2135, 215-28-3 having the Accession No. I-2136, 534-16 having the Accession No. I-2137 and 606-8-11-67 having the Accession No. I-2138."

CLAIMS:

1. A monoclonal antibody (Mab) isolated using a two-Mab sandwich enzyme-linked immunosorbant assay (ELISA) and capable of binding Placental Protein 13 (PP-13).

2. A Mab according to Claim 1 produced by a hybridoma cell selected from the group consisting of clones # 26-2, 27-2-3, 215-28-3, 534-16 and 606-8-11-67.

3. A hybridoma clone selected from the group consisting of clones # 26-2, 27-2-3, 215-28-3, 534-16 and 606-8-11-67.

4. An immunoassay for measuring the level of PP-13 in a biological fluid comprising the steps of:

(a) bringing said fluid into contact with a Mab according to Claim 1, thereby forming Mab-PP-13 complexes;

(b) exposing said complexes to a second antibody linked to a signal-generating molecule, said second antibody being capable of binding said complexes, wherein said second antibody is also a Mab according to Claim 1; and

(c) providing conditions conducive to the production of a signal generated by said signal-generating molecule.

5. An immunoassay according to Claim 4 wherein said Mab in step (a) is bound to a solid phase.

6. An immunoassay according to Claim 4 wherein said signal generating molecule is an enzyme.

7. An immunoassay according to Claim 4 wherein said signal generating molecule is a ligand, and step (c) of claim 4 comprises incubating the product of step (b) with a ligand binding molecule linked to an enzyme.

8. An immunoassay according to Claim 7 wherein said ligand is biotin and said ligand-binding molecule is extravidin.

9. A kit for measuring the level of PP-13 in a biological fluid comprising

(a) a Mab according to Claim 1;

(b) a second antibody linked to a signal-generating molecule wherein said second antibody is also a Mab according to Claim 1; and

(c) PP-13 standard solutions.

10. A kit according to Claim 9 wherein said Mab in step (a) is bound to a solid phase.

11. A kit according to Claim 9 wherein said signal generating molecule is an enzyme.

12. A kit according to Claim 9 wherein said signal generating molecule is a ligand, and said kit further comprises a ligand binding molecule linked to an enzyme.

13. A kit according to Claim 12 wherein said ligand is biotin and said ligand-binding molecule is extravidin.

INTERNATIONAL SEARCH REPORT

Interi .nal Application No
PCT/IL 00/00196

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K16/18 C12N5/20 G01N33/577 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K C12N G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, MEDLINE, BIOSIS, CHEM ABS Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 198 366 A (SILBERMAN) 30 March 1993 (1993-03-30) claims 1-13	1-15
A	US 4 500 451 A (BOHN ET AL) 19 February 1985 (1985-02-19) claims 1-3	1-15
P,A	WO 99 38970 A (DIAGNOSTIC TECHNOLOGIES) 5 August 1999 (1999-08-05) claims 1-11	1-15

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 June 2000	12/07/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Le Flao, K

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inte ional Application No
PCT/IL 00/00196

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,A	THAN N ET AL: "Isolation and sequence analysis of a cDNA encoding human placental tissue protein 13 (PP13), a new lysophospholipase, homologue of human eosinophil Charcot-Leyden crystal protein" PLACENTA (NOV. 1999) 20 (8) 703-10, XP000915077 abstract	1-15

1

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

14

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
124100.9 SZ		
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Date of submission of the demand	Date of completion of this report
19/10/2000	26.06.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Herrero, M Telephone No. +49 89 2399 8542

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/IL00/00196

I. Basis of the report

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Novelty (N)	Yes:	Claims	1-13
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-13
Industrial applicability (IA)	Yes:	Claims	1-13
	No:	Claims	

2. Citations and explanations
   see separate sheet

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see separate sheet

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According to the supporting description, the hereby characterized anti-PP-13 Mabs capable of binding with high affinity/specificity PP13 (i.e. the Mabs obtainable from one of the following hybridoma clones: # 26-2 having the Accession No. I-2134, # 27-2-3 having the Accession No I-2135, # 215-28-3 having the Accession No. I-2136, # 534-16 having the Accession No. I-2137 or # 606-8-11-67 having the Accession No. I-2138) were identified/selected by means of sequential screening assays involving (i) an antibody capture ELISA (cf page 8, lines 27-29 bridging over page 9, lines 1-8 and page 11, lines 8-16) followed by (ii) a two antibody sandwich ELISA employing a rabbit polyclonal anti-PP-13 IgG as a primary Ab and antisera of test bleeds, hybridoma culture supernatants or ascitic fluids as the secondary Abs (cf page 9, lines 12-19 and page 11, lines 17-24).

The selected Mabs thus identified (i.e. the intended Mabs according to present Claim 2) were therefore not "isolated using a two-Mab sandwich enzyme-linked immunosorbant assay (ELISA)". Accordingly, the amendment introduced in the newly filed Claim 1 contravenes Article 34(2)(b) PCT.

In connection to the foregoing it is noted that, subsequently to their identification/isolation, the potential applicability of several Mabs of interest (i.e. of the intended Mabs according to present Claim 2) as components of a two-Mab sandwich ELISA for the PP13 was tested. As stated in the supporting description on page 12, lines 13-16 "*Two-antibody sandwich assays with different combinations of primary and secondary Ab were carried out. The most effective*

variant was found to use IgG # 27-2-3 for coating and Biotin-IgG # 215-28-3 as a secondary Ab (Fig. 11)" (see also page 9, lines 21-29 bridging over page 10, lines 1-22).

6. Additional observations

This preliminary examination report takes into consideration the content of the Applicants' letter of 31.05.01 in reply to the written opinion dated 04.01.01, as well as the additional technical information enclosed therein.

SECTION V

2. CITATIONS AND EXPLANATIONS

2.1 In view of the priority documents pertaining to the present application, the international patent application WO 99/38970 (publication date 05.08.99) and the scientific publication by Than, N.G. *et al* in Placenta **20**:703-710 (November 99), cited in the International Search Report under the "P" category, are not to be regarded as state of the art according to Rule 64 (1) PCT as the date of priority of 30.03.99 is hereby validly claimed.

Nevertheless, the aforementioned document WO 99/38970 (filed on 21.01.99), which describes monoclonal antibodies falling under the scope of present Claim 1 (cf page 4, lines 1-3), is brought to the Applicant's attention in view of the provisions of Article 54(3)(4) EPC.

2.2 The following documents have been considered for the purposes of this report:

D1: US-A-5198366 (also cited in the application)
D2: US-A-4500451 (also cited in the application)

D1 discloses a radioimmunoassay (RIA) and an enzyme-linked immunosorbent assay (ELISA) based on the use of labelled human placental protein 13 (PP13) and anti-PP13 polyclonal antiserum, respectively. Both assays were developed

with the purpose of enabling the detection of three specific pregnancy-related disorders: intrauterine growth retardation, preeclampsia and preterm delivery (cf column 2, lines 57-63; column 3, lines 45-68-column 4-column 5, line 1; Claims 2 and 7).

The diagnostic significance of PP13 had been previously established, e.g. in D2 (cf column 4, lines 36-47). In addition to the data related to the isolation and characterization of human PP13, D2 also teaches the immunological determination of PP13 based on the use of monospecific antisera prepared by immunizing animals (e.g. rabbits) by known methods using the purified PP13 obtained by the procedure therein disclosed (cf column 4, lines 2-22).

2.3 The newly filed Claims 1 to 13 appear to relate to subject-matter which is novel over the available prior art, as required by Art. 33(2) PCT.

The arguments defending the non-obviousness of Claims 1 to 13 put forward by the Applicants in the aforementioned letter of 31.05.01 have been taken into account. However it is still considered that the application does not satisfy the criterion set forth in Art. 33(3) PCT because the subject-matter presently claimed does not involve an inventive step (Rule 65(1)(2) PCT).

In view of the related prior art (e.g. D1 and D2 above), the objective problem solved by the claimed invention according to Claim 1 (Mabs), Claims 4-8 (immunoassays) and Claims 9-13 (kits) merely relates to the provision of Mabs raised against PP13 and kits containing the same, and their use in an immunoassay for measuring the level of PP13 in biological samples (see also page 3, lines 2-5 of the application).

To serve the above purpose the application characterizes (and seemingly renders available by means of deposit) five different Mabs. These Mabs are identified in the description (and in Claims 2 and 3) by the arbitrary numerical denominations assigned to the specific producing clones which are listed in the Table shown on page 3 (accession Nos. corresponding to deposited hybridomas producing each one of the five Mabs are additionally shown in said Table).

Taking into account the technical advances in the fields of immunology and/or biotechnology achieved at the priority date of the present application, it is apparent that the preparation of Mabs directed against the well-known protein antigen PP13, which had been already purified and used for the generation of specific polyclonal antibodies (see e.g. D1 and D2), would have not required from the person skilled in the art the practice of any inventive activity.

In the absence of evidence demonstrating the contrary it has to be assumed that, since the successful generation of monospecific antisera/polyclonal antibodies against PP13 had been previously disclosed, the preparation of corresponding Mabs (i.e. the Mabs of Claim 1) would have been obvious to the skilled person. Thus no inventive contribution is recognisable in claiming the generic Mab capable of binding PP13 according to Claim 1.

As a consequence of the above major objection Claims 4-8, encompassing conventional immunoassay procedures for measuring the level of PP-13 in a biological fluid (i.e. under standard sandwich ELISA formats employing two Mabs), all of them relying on the use of the non-inventive Mabs of Claim 1, are also objected to under Art. 33(3) PCT due to lack of inventive step.

Moreover, insofar as the packaging of the components necessary for the performance of a known or obvious assay (e.g. the ELISA of D1) into a kit is an obvious application of such an assay, present Claims 9-13, directed to kits for measuring the level of PP13 in a biological fluid, which rely of the use of the non-inventive Mabs of Claim 1 [as components (a) and (b) thereof] cannot be considered inventive, contrary to Art. 33(3) PCT.

With regard to the five specific Mabs obtainable from the deposited hybridoma cell lines with the Accession Nos. I-2134 to I-2138, it is presently not evident on which grounds they (i.e. all of them) should *per se* be acknowledged as inventive.

On the one hand, the preparation of generic Mabs directed against PP13 is considered to be straightforward for the skilled person, vis-à-vis D1 or D2 (see

above discussion). On the other hand, having regard to the supporting description on page 11, lines 7-29 bridging over page 12, lines 1-5, the selection of the five specific clones producing the aforementioned Mabs of interest seems to have been exclusively based on standard sequential screening procedures aimed at identifying several stable clones producing high affinity Abs (see the third paragraph of item 5 in the above Section I). These screening procedures, which were carried out under conventional conditions and making use of well-known components, i.e. the purified antigen PP13 and a rabbit anti-PP13 polyclonal Ab (apparently equivalent to the first Ab employed in D1), merely represent a trial-and-error approach which comes within the scope of the customary practice followed by persons skilled in the art, especially as the advantages thus achieved, i.e. the identification of particular clones producing Mabs potentially useful to carry out an improved version of the ELISA assays of D1, can readily be foreseen.

From the results presented in Figures 4, 5 and 11 it could be concluded that only the Mab produced by the selected clone denominated # 215-28-3 would be suitable to solve the underlying technical problem of efficiently differentiating in a biological fluid of interest (e.g. blood serum) those distinctive levels of PP-13 characteristic of/associated with pregnancy/pregnancy disorders (cf page 14, lines 18-24 bridging over page 15, lines 1-2). Conversely, it is not evident from the results presented in Figures 4, 5 and 11 that the rest of the specific Mabs claimed in Claim 2 could solve the problem posed.

Under these circumstances, the subject-matter of Claims 2 and 3 would not appear to satisfy the requirements of Art. 33(3) PCT as these claims respectively encompass every one of the aforementioned five specific Mabs and their corresponding producing hybridomas.

2.4 Notwithstanding the foregoing it would appear that the application contains subject-matter which is novel and inventive over the prior art and industrially applicable, as required by Art. 33(2)(3) and (4) PCT. In this regard the application seemingly enables a highly sensitive (i.e. 0.05 ng/ml of PP-13) two-Mab sandwich ELISA (cf page 12, lines 12-17) based on the use of IgG # 27-2-3 for coating (primary Mab) and Biotin-IgG # 215-28-3 as a secondary Mab (it is assumed that the advantageous results concerning sensitivity and specificity of the two-Mab

sandwich ELISA test presented in Figures 13 and 14 have been obtained employing said combination of primary and secondary Mabs, wherein the second Mab is produced by the selected clone denominated # 215-28-3).

(It is also assumed that the "Mab of the invention" employed to generate the comparative data provided by the Applicants is either the Mab produced by the selected clone denominated # 215-28-3 or a anti-PP-13 Mab with equivalent behaviour).

SECTION VII

1. The terms " Tween " (page 6, line 11 and page 10, lines 4 and 5), and "Superdex" and "Hiload" (page 7, line 14) appear to be registered trade marks, but have not been acknowledged as such.

2. The expression "hereby incorporated by reference" in respect of prior art documents on page 1, lines 14-15 and page 2, lines 3-4, leads to a doubt as to whether the requirements of the description being self-contained are satisfied (see PCT Guidelines C-II, 4-17).

SECTION VIII

The arbitrary numerical denominations given in Claims 2 and 3 to the intended hybridoma cell clones therein referred to are *per se* devoid of technical significance and leave the reader in doubt as to the actual meaning of the essential characterizing feature to which it refers, thereby rendering the definition of the subject-matter of said claims unclear (Article 6 PCT).

In order to overcome this deficiency (each one of) the clones mentioned in Claims 2 and 3 should have been clearly identified reciting, for instance, "... hybridoma ... // ... selected from the group of clones # 26-2 having the Accession No. I-2134, 27-2-3 having the Accession No I-2135, 215-28-3 having the Accession No. I-2136, 534-16 having the Accession No. I-2137 and 606-8-11-67 having the Accession No. I-2138."

COURTESY COPY OF THE PCT APPLICATION AS ORIGINALLY FILED WITH ABSTRACT

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
124100.9 SZ		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/IL 00/00196	29/03/2000	30/03/1999
Applicant		
DIAGNOSTIC TECHNOLOGIES LTD. et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
[redacted]/IL 00/00196

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K16/18 C12N5/20 G01N33/577 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K C12N G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, MEDLINE, BIOSIS, CHEM ABS Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 198 366 A (SILBERMAN) 30 March 1993 (1993-03-30) claims 1-13	1-15
A	US 4 500 451 A (BOHN ET AL) 19 February 1985 (1985-02-19) claims 1-3	1-15
P,A	WO 99 38970 A (DIAGNOSTIC TECHNOLOGIES) 5 August 1999 (1999-08-05) claims 1-11	1-15

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 June 2000	12/07/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Le Flao, K

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,A	THAN N ET AL: "Isolation and sequence analysis of a cDNA encoding human placental tissue protein 13 (PP13), a new lysophospholipase, homologue of human eosinophil Charcot-Leyden crystal protein" PLACENTA (NOV. 1999) 20 (8) 703-10, XP000915077 abstract	1-15

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
[redacted]/IL 00/00196

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
US 5198366	A	30-03-1993	IL	78237 A	30-06-1991
			CA	1295939 A	18-02-1992
US 4500451	A	19-02-1985	DE	3230996 A	23-02-1984
			AT	38521 T	15-11-1988
			AU	555699 B	02-10-1986
			AU	1816783 A	23-02-1984
			CA	1213213 A	28-10-1986
			DE	3378414 D	15-12-1988
			EP	0101603 A	29-02-1984
			JP	1693459 C	17-09-1992
			JP	3054680 B	20-08-1991
			JP	59059621 A	05-04-1984
WO 9938970	A	05-08-1999	AU	2071799 A	16-08-1999

CLAIMS:

1. A monoclonal antibody (Mab) capable of binding Placental Protein 13 (PP-13).

2. A Mab according to Claim 1 produced by a hybridoma cell selected from the group consisting of clones # 26-2, 27-2-3, 215-28-3, 534-16 and 606-8-11-67.

3. A hybridoma clone selected from the group consisting of clones # 26-2, 27-2-3, 215-28-3, 534-16 and 606-8-11-67.

4. An immunoassay for measuring the level of PP-13 in a biological fluid comprising the steps of:

(a) bringing said fluid into contact with a Mab according to Claim 1, thereby forming Mab-PP-13 complexes:

(b) exposing said complexes to a second antibody linked to a signal-generating molecule, said second antibody being capable of binding said complexes; and

(c) providing conditions conducive to the production of a signal generated by said signal-generating molecule.

5. An immunoassay according to Claim 4 wherein said Mab in step (a) is bound to a solid phase.

6. An immunoassay according to Claim 4 wherein said second antibody is also a Mab according to Claim 1.

7. An immunoassay according to Claim 4 wherein said signal generating molecule is an enzyme.

8. An immunoassay according to Claim 4 wherein said signal generating molecule is a ligand, and step (c) of claim 4 comprises incubating the product of step (b) with a ligand binding molecule linked to an enzyme.

9. An immunoassay according to Claim 8 wherein said ligand is biotin and said ligand-binding molecule is extravidin.

10. A kit for measuring the level of PP-13 in a biological fluid comprising

(a) a Mab according to Claim 1;

(b) a second antibody linked to a signal-generating molecule; and

(c) PP-13 standard solutions.

11. A kit according to Claim 10 wherein said Mab in step (a) is bound to a solid phase.

12. A kit according to Claim 10 wherein said second antibody is also a Mab according to Claim 1.

13. A kit according to Claim 10 wherein said signal generating molecule is an enzyme.

14. A kit according to Claim 10 wherein said signal generating molecule is a ligand, and said kit further comprises a ligand binding molecule linked to an enzyme.

15. A kit according to Claim 14 wherein said ligand is biotin and said ligand-binding molecule is extravidin.